

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/002522

International filing date: 26 January 2005 (26.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
 Number: 60/626,678
 Filing date: 09 November 2004 (09.11.2004)

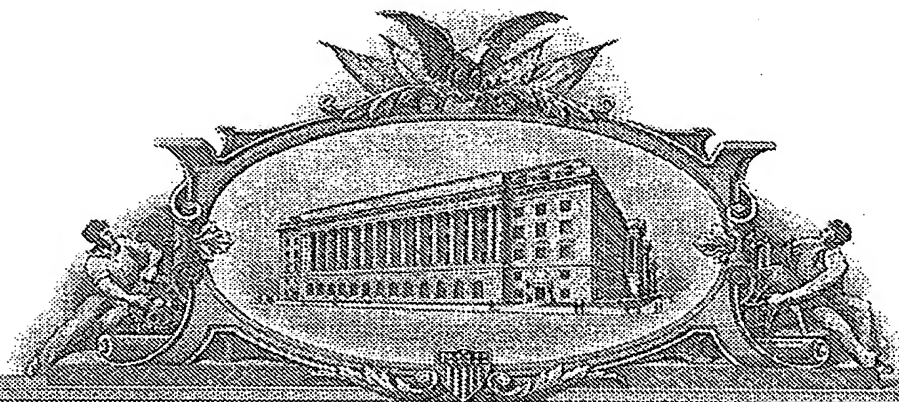
Date of receipt at the International Bureau: 31 March 2005 (31.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1799001



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

March 21, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/626,678

FILING DATE: November 09, 2004

RELATED PCT APPLICATION NUMBER: PCT/US05/02522



Certified by

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 520328363US

21861 U.S. PTO

110904

17548 U.S. PTO
60/626678

110904

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Shawn	DeFrees	North Wales, PA			
Zhi-Guang	Wang	Dresher, PA			
<input type="checkbox"/> Additional inventors are being named on the separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
GLYCOLIPIDS					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number		043850			
OR					
<input type="checkbox"/> Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		51	<input type="checkbox"/> CD(s), Number		
<input type="checkbox"/> Drawing(s) Number of Sheets			<input type="checkbox"/> Other (specify)		
<input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.					
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0310		Payment by credit card. Form PTO-2038 is attached.		FILING FEE Amount (\$)	80.00
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:					

Respectfully submitted,

[Page 1 of 2]

Date 11/09/04

SIGNATURE

REGISTRATION NO. 46,690
(if appropriate)

TYPED or PRINTED NAME Todd Esker

Docket Number: 040853-01-5157-PR

TELEPHONE (415) 442-1304

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Attorney Docket No.: 040853-01-5157-PR

Client Reference No.: NEO00320

PROVISIONAL

PATENT APPLICATION

GLYCOLIPIDS

Inventor(s): Shawn DeFrees, a citizen of The United States, residing at
126 Filly Drive
North Wales, PA 19454

Zhi-Guang Wang, a citizen of The United States
and The Peoples Republic of China,
residing at
1473 Golden Drive
Dresher, PA 19025

Assignee: Neose Technologies, Inc.
102 Witmer Road
Horsham, PA 19044

Entity: Small

Todd Esker
Reg. No. 46,690

MORGAN
LEWIS AND
BOCKIUS
LLP

Correspondence Address:

One Market
Spear Street Tower
San Francisco
California 94105
Tel 415 442-1304
Fax 415 442-1000

AS FILED IN THE USPTO ON Tuesday, November 09, 2004

GLYCOLIPIDS

BACKGROUND OF THE INVENTION

5 [0001] Gangliosides are glycosphingolipids, often found in cell membranes, that consist of three elements. One or more sialic acid residues are attached to an oligosaccharide or carbohydrate core moiety, which in turn is attached to a hydrophobic lipid (ceramide) structure which generally is embedded in the cell membrane. The ceramide moiety includes a long chain base (LCB) portion and a fatty acid (FA) portion. Gangliosides, as well as other glycolipids and their structures in 10 general, are discussed in, for example, Lehninger (Biochemistry, Freeman, 4th Ed., 343-369 (2004)) and Devlin (Textbook of Biochemistry, Wiley-Liss, (1992)). Gangliosides are classified according to the number of monosaccharides in the carbohydrate moiety, as well as the number and location of sialic acid groups present 15 in the carbohydrate moiety. Mono sialogangliosides are given the designation "GM", disialogangliosides are designated "GD", trisialogangliosides "GT", and tetrasialogangliosides are designated "GQ". Gangliosides can be classified further depending on the position or positions of the sialic acid residue or residues bound. Further classification is based on the number of saccharides present in the 20 oligosaccharide core, with the subscript "1" designating a ganglioside that has four saccharide residues (Gal-GalNAc-Gal-Glc-Ceramide), and the subscripts "2", "3" and "4" representing trisaccharide (GalNAc-Gal-Glc-Ceramide), disaccharide (Gal-Glc-Ceramide) and monosaccharide (Gal-Ceramide) gangliosides, respectively.

[0002] Numerous types of gangliosides found in nature have been isolated and 25 identified and vary primarily in the basic saccharide structure (e.g. GM3, GM2, GM1, GD1a, GD1b and GT1). A variety of procedures are available for the isolation and purification of such "natural" gangliosides from organs and tissues, particularly from animal brain (Sonnino *et al.*, *J. Lipid Res.*, **33**:1221-1226 (1992); Sonnino *et al.*, *Ind. J. Biochem. Biophys.*, **25**:144-14 (1988); Svennerholm, *Adv. Exp. Med. Biol.*, **125**:533-44 (1980)) as well as bovine buttermilk (Ren *et al.*, *J. Bio. Chem.*, **267**:12632-12638 (1992); Takamizawa *et al.*, *J. Bio. Chem.*, **261**:5625-5630 (1986)). 30

[0003] Gangliosides are normal components of plasma membranes and are particularly abundant in the nervous system. In humans, gangliosides are most abundant in the gray matter of the brain, particularly in nerve endings. They are believed to be present at receptor sites for neurotransmitters, including acetylcholine,
5 and can also act as specific receptors for other biological macromolecules, including interferon, hormones, viruses, bacterial toxins, and the like.

[0004] Certain gangliosides are found on the surface of human hematopoietic cells (Hildebrand *et al.*, *Biochim. Biophys. Acta*, **260**: 272-278 (1972); Macher *et al.*, *J. Biol. Chem.* **256**:1968-1974 (1981); Dacremont *et al.*, *Biochim. Biophys. Acta*
10 **424**:315-322; Klock *et al.*, *Blood Cells* **7**:247 (1981)) which may play a role in the terminal granulocytic differentiation of these cells. Nojiri *et al.*, *J Biol. Chem.* **263**:7443-7446 (1988)). These gangliosides, referred to as the "neolacto" series, have neutral core oligosaccharide structures having the formula
[Gal β -(1,4)GlcNAc β (1,3)]_nGal β (1,4)Glc, where n = 1-4. Included among these
15 neolacto series gangliosides are 3'-nLM₁
(NeuAc α (2,3)Gal β (1,4)GlcNAc β (1,3)Gal β (1,4)-Glc β (1,1)-Ceramide) and 6'-nLM₁
(NeuAc α (2,6)Gal β (1,4)GlcNAc β (1,3)Gal β (1,4)-Glc β (1,1)-Ceramide).

[0005] It has been widely demonstrated that gangliosides are able to enhance functional recovery both in the lesioned peripheral nervous system (PNS) and the
20 central nervous system (CNS), through the involvement of specific membrane mechanisms and the interaction with trophic factors, as pointed out from studies *in vitro* on neuronal cultures (Ferrari, F. *et al.*, *Dev. Brain Res.*, **8**:215-221 (1983); Doherty, P. *et al.*, *J. Neurochem.*, **44**:1259-1265 (1985); Skaper, S.D. *et al.*, *Mol. Neurobiol.*, **3**:173-199 (1989)). Gangliosides have been used for treatment of nervous
25 system disorders, including cerebral ischemic strokes. See, *e.g.*, Mahadnik *et al.*, *Drug Development Res.*, **15**:337-360 (1988); US Patent Nos. 4,710,490 and 4,347,244; Horowitz, *Adv. Exp. Med. and Biol.*, **174**:593-600 (1988); Karpitz *et al.*, *Adv. Exp. Med. and Biol.*, **174**:489-497 (1984).

[0006] As a result, attempts have been made to use gangliosides in the treatment
30 of disorders of the nervous system. This has led to the development of synthetic gangliosides as well as natural ganglioside containing compositions for use in the treatment of disorders of the nervous system (U.S. Pat. Nos. 4,476,119, 4,593,091,

4,639,437, 4,707,469, 4,713,374, 4,716,223, 4,849,413, 4,940,694, 5,045,532, 5,135,921, 5,183,807, 5,190,925, 5,210,185, 5,218,094, 5,229,373, 5,260,464, 5,264,424, 5,350,841, 5,424,294, 5,484,775, 5,519,007, 5,521,164, 5,523,294, 5,677,285, 5,792,858, 5,795,869, and 5,849,717).

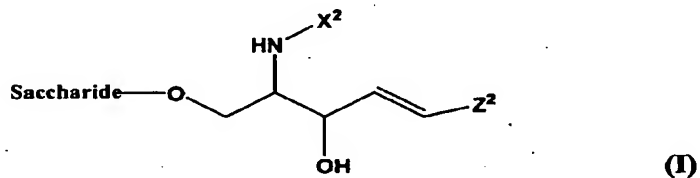
- 5 [0007] Gangliosides have also been implicated as playing a significant role in certain types of cancer. Neuroblastoma is a form of cancer that primarily afflicts children under the age of five. Individuals suffering from neuroblastoma may have tumors growing near the spinal cord, and very large tumors have been found to cause paralysis in such patients. Gangliosides have been shown to play a role in both the
- 10 growth and the inhibition of the growth of neuroblastoma-associated tumors (Basavarajappa *et al.*, *Alcohol Clin. Exp. Res.*, 21(7):1199-1203 (1997); Singleton *et al.*, *Int. J. Dev. Neurosci.*, 18(8):797-780 (2000)).

- [0008] However, there still exists a need in the art for compounds capable of acting as neuroprotective agents in a manner similar to or better than the natural
- 15 gangliosides for the prophylaxis, treatment and cure of disorders of the nervous system. Further, differences in the structure of ganglioside compounds can refine the structure-function relationship of such compounds to provide powerful tools for control of the growth of certain kinds of tumors, including neuroblastoma tumors.

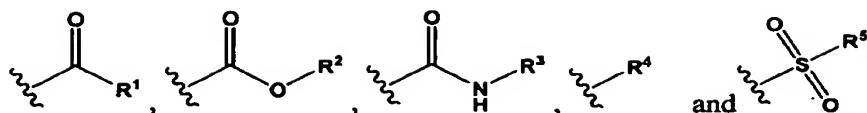
SUMMARY OF THE INVENTION

- 20 [0009] The present invention answers this need by providing novel synthetic gangliosides of formulae (I)-(III), which are listed below.

[0010] The invention further provides a novel synthetic ganglioside according to Formula (I):

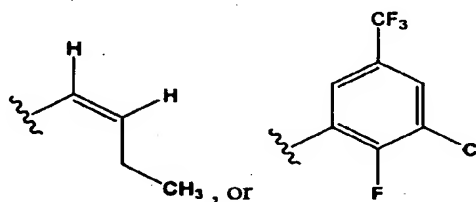


- 25 in which X² can be

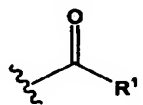


In the formulae above, R^1 , R^2 , R^3 , R^4 , and R^5 can be independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl. Z^2 can be substituted or unsubstituted alkenyl, substituted or unsubstituted pyridinyl, and substituted or unsubstituted phenyl.

[0011] The present invention also provides a novel ganglioside as described above, with the proviso that when R^1 is $-(CH_2)_{16}-CH_3$, then Z^2 is not unsubstituted pyridinyl,

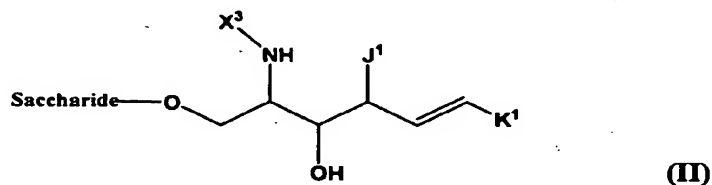


[0012] In another embodiment, X^2 can be

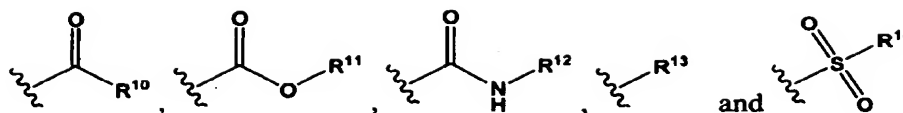


In the formula above, R^1 is substituted or unsubstituted alkyl. In yet another embodiment, R^1 can be a substituted or unsubstituted C_8-C_{20} alkyl moiety. In still another embodiment, wherein R^1 can be haloalkyl. In another embodiment, R^1 can be a member selected from chloromethyl and dichloromethyl.

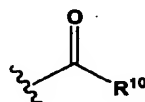
[0013] The invention further provides a novel synthetic ganglioside according to Formula (II):



20 in which X^3 can be

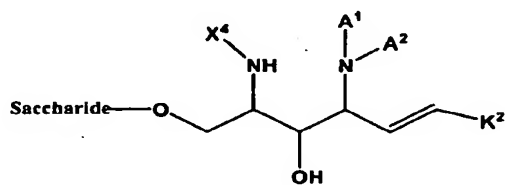


In the formulae above, R^{10} , R^{11} , R^{12} , R^{13} , and R^{14} can be members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl. J^1 can be $-NR^{15}R^{16}$, in which R^{15} and R^{16} can be members independently selected from substituted or unsubstituted alkyl. K^1 can be a member selected from substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. In another embodiment, at least one member selected from R^{15} and R^{16} can be $-(CH_2)_7-CH_3$. In a further embodiment, K^1 can be substituted or unsubstituted aryl. In yet another embodiment, K^1 can be substituted or unsubstituted phenyl. In still another embodiment, X^3 can be



In the formula above, R^{10} can be substituted or unsubstituted alkyl. In yet another embodiment, R^{10} can be a substituted or unsubstituted alkyl moiety. In another embodiment, R^{10} can be haloalkyl. In still another embodiment, R^{10} can be a member selected from chloromethyl and dichloromethyl.

[0014] The invention further provides a novel synthetic ganglioside according to Formula (III):



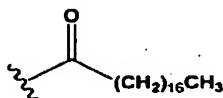
(III)

In the formula above, X^4 can be substituted or unsubstituted alkyl, $-C(=M^2)R^{20}$, $-C(=M^2)-Q^2-R^{20}$, $-SO_2R^{20}$. R^{20} can be a member selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. M^2 and Q^2 can be independently selected from O, NR^{21} and S. R^{21} can be H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted

heterocycloalkyl. A^1 and A^2 can be independently selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl. K^2 can be substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl.

[0015] The present also provides a novel ganglioside compound as described above, with the proviso that if A^1 is $-(CH_2)_7-CH_3$, A^2 is $-(CH_2)_7-CH_3$, X^4 is $-C(=O)R^{20}$, and R^{20} is $-(CH_2)_{16}-CH_3$, then K^2 is not unsubstituted phenyl.

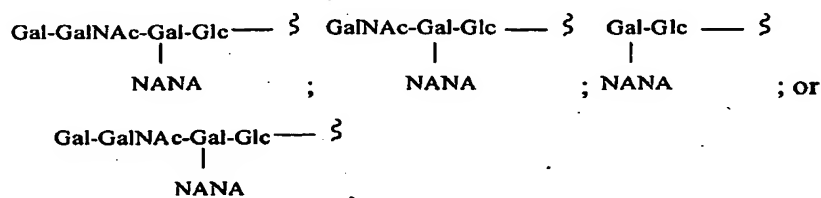
[0016] In another embodiment of the invention, X^4 can be $-C(=O)R^{20}$, and R^{20} can be substituted or unsubstituted alkyl. In yet another embodiment of the invention, X^4 can be



In yet another embodiment, A^1 and A^2 can be independently selected from substituted or unsubstituted alkyl. In still another embodiment, A^1 and A^2 can be $-(CH_2)_7-CH_3$.

In another embodiment, K^2 can be substituted or unsubstituted aryl. In still another embodiment, K^2 can be phenyl.

[0017] The present invention also provides a novel ganglioside compound in which the saccharide component can be



and such saccharide moieties may or may not be deacetylated.

[0018] The invention further provides pharmaceutical compositions including at least one compound of the invention and a pharmaceutically acceptable carrier.

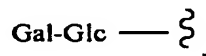
[0019] The invention still further provides a method for the prevention and/or treatment and/or cure of a disorder of the nervous system in an animal or human including the step of administering to a patient in need thereof a therapeutically

effective amount of at least one compound or pharmaceutical composition of the invention. Such patients in need of a compound of the present invention may suffer from a disorder of the nervous system, including Parkinson's disease, ischemia, stroke, Alzheimer's disease, depression, anxiety, encephalitis, meningitis,

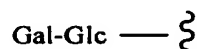
5 amyotrophic lateral sclerosis, trauma, spinal cord injury, nerve injury, and nerve regeneration.

[0020] One embodiment of the invention provides a method for the treatment of a glioma in a human and includes the step of administering to the human in need thereof a therapeutically effective amount of a compound of the present invention.

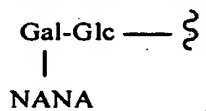
10 [0021] The present invention also provides a method of synthesizing a synthetic ganglioside compound of the invention, wherein the steps of synthesis of the saccharide moiety include contacting a sphingoid acceptor molecule and a glucose molecule with a galactosyltransferase enzyme and a galactose donor molecule to form



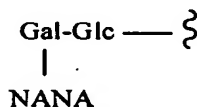
15 contacting the



with a trans-sialidase enzyme and a sialic acid (NANA) donor molecule to form

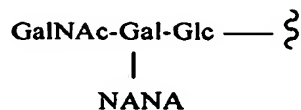


contacting the

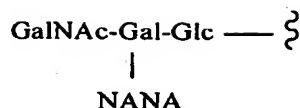


20

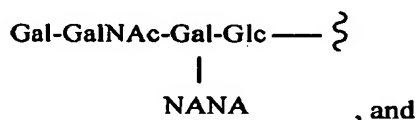
with a N-acetyl galactose (GalNAc)-transferase enzyme and a GalNAc donor molecule to form



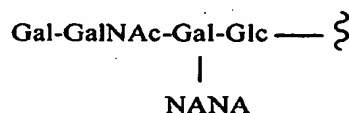
contacting the



with a galactosyltransferase enzyme and a galactose (Gal) donor molecule to form



contacting the



5

with a fatty acid moiety under conditions sufficient to form a ganglioside.

DETAILED DESCRIPTION OF THE INVENTION

A. Introduction

10 [0022] Novel synthetic ganglioside compositions are described herein. Following a discussion of the ganglioside compositions, methods of making the synthetic ganglioside compositions, pharmaceutical compositions containing such synthetic gangliosides, and methods of using the synthetic ganglioside compositions as neuroprotecting agents are also presented.

B. Definitions

15 [0023] In accordance with the invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

[0024] The article "a" and "an" as used herein refers to one or to more than one (*i.e.* at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

20 [0025] The term "alkenyl" as used herein refers to a substituted or unsubstituted trivalent straight chain or branched chain unsaturated aliphatic radical that includes at least two carbons joined by a double bond.

[0026] The term "alkynyl" as used herein refers to a straight or branched chain aliphatic radical that includes at least two carbons joined by a triple bond. If no

number of carbons is specified, "alkenyl" and "alkynyl" each refer to radicals having from 2-12 carbon atoms.

[0027] The term "cycloalkyl" as used herein refers to a substituted or unsubstituted saturated aliphatic ring system, preferably a mono-, bi-, or tricyclic saturated aliphatic ring system. Examples include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, adamantyl, cyclooctyl, [3.3.0]bicyclooctane, [4.3.0]bicyclononane, [4.4.0]bicyclodecane (decalin), and [2.2.2]bicyclooctane.

[0028] The term "aromatic" is intended to mean stable substituted or unsubstituted mono-, bi-, tri-, polycyclic ring structures having only carbon atoms as ring atoms including, but not limited to, a stable monocyclic ring which is aromatic having six ring atoms; a stable bicyclic ring structure having a total of from 7 to 12 carbon atoms in the two rings of which at least one of the rings is aromatic; and a stable tricyclic ring structure having a total of from 10 to 16 atoms in the three rings wherein the tricyclic ring structure of which at least one of the ring is aromatic. Any non-aromatic rings present in the monocyclic, bicyclic, tricyclic or polycyclic ring structure may independently be saturated, partially saturated or fully saturated. Examples of such "aromatic" groups include, but are not limited to, phenyl and naphthyl.

[0029] The term "arylalkyl" as used herein refers to one, two, or three substituted or unsubstituted aryl groups having the number of carbon atoms designated appended to an alkyl group having the number of carbon atoms designated. The direction of attachment of an arylalkyl group to the remainder of the molecule may be through either the aryl or alkyl portion of the group. Suitable arylalkyl groups include, but are not limited to, benzyl, picolyl, naphthylmethyl, phenethyl, benzylhydryl, trityl, and the like, all of which may be optionally substituted.

[0030] As used herein the term "heteroaryl," "heteroaromatic" or "aromatic heterocyclic ring system" refers to a monocyclic, bicyclic or polycyclic, substituted or unsubstituted heterocyclic ring system containing at least one aromatic ring.

[0031] The term "substituted" as used herein means that a hydrogen atom has been replaced with another monovalent group (e.g. halo, haloalkyl, hydroxy, thiol, alkoxy, thiohaloalkyl, amino, and the like).

[0032] The terms "halo" or "halogen" as used herein refer to Cl, Br, F or I. The term "haloalkyl" and the like, refer to an alkyl group, as defined herein, wherein at least one hydrogen atom of the alkyl group is replaced by a Cl, Br, F or I. A mixture of different halo atoms may be used if more than one hydrogen atom is replaced. For example, a haloalkyl includes chloromethyl ($-\text{CH}_2\text{Cl}$) and trifluoromethyl ($-\text{CF}_3$) and the like.

[0033] The term "methylene" refers to $-\text{CH}_2-$.

[0034] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents which would result from writing the structure from right to left, *e.g.*, $-\text{CH}_2\text{O}-$ is intended to also recite $-\text{OCH}_2-$.

[0035] The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain, or cyclic hydrocarbon radical, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (*i.e.* $\text{C}_1\text{-C}_{10}$ means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *t*-butyl, isobutyl, *sec*-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers. The term "alkyl," unless otherwise noted, is also meant to include those derivatives of alkyl defined in more detail below, such as "heteroalkyl." Alkyl groups which are limited to hydrocarbon groups are termed "homoalkyl".

[0036] The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$, and further includes those groups described below as "heteroalkylene." Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred in

the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

[0037] The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl groups attached to the remainder
5 of the molecule via an oxygen atom, an amino group, or a sulfur atom, respectively.

[0038] The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or cyclic hydrocarbon radical, or combinations thereof, consisting of the stated number of carbon atoms and at least one heteroatom selected from the group consisting of O, N,
10 Si and S, and wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to, -CH₂-CH₂-O-CH₃, -CH₂-CH₂-NH-CH₃, -
15 CH₂-CH₂-N(CH₃)-CH₃, -CH₂-S-CH₂-CH₃, -CH₂-CH₂-S(O)-CH₃, -CH₂-CH₂-S(O)₂-CH₃, -CH=CH-O-CH₃, -Si(CH₃)₃, -CH₂-CH=N-OCH₃, and -CH=CH-N(CH₃)-CH₃. Up to two heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃ and -CH₂-O-Si(CH₃)₃. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified,
20 but not limited by, -CH₂-CH₂-S-CH₂-CH₂- and -CH₂-S-CH₂-CH₂-NH-CH₂-. For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxy, alkylenedioxy, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group
25 is written. For example, the formula -C(O)₂R'- represents both -C(O)₂R'- and -R'C(O)₂-.

[0039] The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a
30 heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like.

Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like.

- 5 [0040] Examples of "heterocycles", "heterocyclic rings" or "heterocyclic ring systems" include, but are not limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazalinyl, carbazolyl, 4aH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl,
- 10 decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolyl, imidazolyl, 1H-indazolyl, indolinyl, indoliziny, indolyl, 3H-indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl (benzimidazolyl), isothiazolyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl,
- 15 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyroazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridoxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl,
- 20 pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinoliziny, quinoxaliny, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl,
- 25 thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl and xanthenyl. Also included are fused ring and spiro compounds containing, for example, the above heterocyclic ring structures.

- [0041] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine
- 30 atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C₁-C₄)alkyl" is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

[0042] The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent which can be a single ring or multiple rings (preferably from 1 to 3 rings) which are fused together or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain from one to four heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxaliny, 5-quinoxaliny, 3-quinolyl, and 6-quinolyl. Substituents for each of the above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below.

[0043] For brevity, the term "aryl" when used in combination with other terms (e.g., aryloxy, arylthioxy, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like).

[0044] Each of the above terms (e.g., "alkyl," "heteroalkyl," "aryl" and "heteroaryl") are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

[0045] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR'C(O)R', -NR'-C(O)NR'R''', -NR'C(O)₂R', -NR-

$C(NR'R''R''')=NR''''$, $-NR-C(NR'R'')=NR''''$, $-S(O)R'$, $-S(O)_2R'$, $-S(O)_2NR'R''$,
 $-NRSO_2R'$, $-CN$ and $-NO_2$ in a number ranging from zero to $(2m'+1)$, where m' is the
total number of carbon atoms in such radical. R' , R'' , R''' and R'''' each preferably
independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted
5 or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or
unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a
compound of the invention includes more than one R group, for example, each of the
 R groups is independently selected as are each R' , R'' , R''' and R'''' groups when more
than one of these groups is present. When R' and R'' are attached to the same
10 nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-
membered ring. For example, $-NR'R''$ is meant to include, but not be limited to, 1-
pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of
skill in the art will understand that the term "alkyl" is meant to include groups
including carbon atoms bound to groups other than hydrogen groups, such as
15 haloalkyl (*e.g.*, $-CF_3$ and $-CH_2CF_3$) and acyl (*e.g.*, $-C(O)CH_3$, $-C(O)CF_3$, $-$
 $C(O)CH_2OCH_3$, and the like).

[0046] Similar to the substituents described for the alkyl radical, substituents for
the aryl and heteroaryl groups are varied and are selected from, for example: halogen,
 $-OR'$, $=O$, $=NR'$, $=N-OR'$, $-NR'R''$, $-SR'$, -halogen, $-SiR'R''R'''$, $-OC(O)R'$, $-C(O)R'$,
20 $-CO_2R'$, $-CONR'R''$, $-OC(O)NR'R''$, $-NR''C(O)R'$, $-NR'-C(O)NR'R'''$, $-$
 $NR''C(O)_2R'$, $-NR-C(NR'R''R''')=NR''''$, $-NR-C(NR'R'')=NR''''$, $-S(O)R'$, $-S(O)_2R'$, $-$
 $S(O)_2NR'R''$, $-NRSO_2R'$, $-CN$ and $-NO_2$, $-R'$, $-N_3$, $-CH(Ph)_2$, fluoro(C_1-C_4)alkoxy,
and fluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open
valences on the aromatic ring system; and where R' , R'' , R''' and R'''' are preferably
25 independently selected from hydrogen, (C_1-C_8)alkyl and heteroalkyl, unsubstituted
aryl and heteroaryl, (unsubstituted aryl)-(C_1-C_4)alkyl, and (unsubstituted aryl)oxy-
(C_1-C_4)alkyl. When a compound of the invention includes more than one R group, for
example, each of the R groups is independently selected as are each R' , R'' , R''' and
 R'''' groups when more than one of these groups is present.

30 **[0047]** Two of the substituents on adjacent atoms of the aryl or heteroaryl ring
may optionally be replaced with a substituent of the formula $-T-C(O)-(CRR')_q-U-$,
wherein T and U are independently $-NR-$, $-O-$, $-CRR'-$ or a single bond, and q is an
integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the

aryl or heteroaryl ring may optionally be replaced with a substituent of the formula –
A-(CH₂)_r-B-, wherein A and B are independently –CRR'-, -O-, -NR-, -S-, -S(O)-,
-S(O)₂-, -S(O)₂NR'- or a single bond, and r is an integer of from 1 to 4. One of the
single bonds of the new ring so formed may optionally be replaced with a double
5 bond. Alternatively, two of the substituents on adjacent atoms of the aryl or
heteroaryl ring may optionally be replaced with a substituent of the formula –
(CRR')_s-X-(CR''R''')_d-, where s and d are independently integers of from 0 to 3, and
X is –O-, -NR'-, -S-, -S(O)-, -S(O)₂-, or –S(O)₂NR'-. The substituents R, R', R'' and
R''' are preferably independently selected from hydrogen or substituted or
10 unsubstituted (C₁-C₆)alkyl.

[0048] As used herein, the term "heteroatom" is meant to include oxygen (O),
nitrogen (N), sulfur (S) and silicon (Si).

[0049] The term "pharmaceutically acceptable salts" is meant to include salts of
the active compounds which are prepared with relatively nontoxic acids or bases,
15 depending on the particular substituents found on the compounds described herein.
When compounds of the present invention contain relatively acidic functionalities,
base addition salts can be obtained by contacting the neutral form of such compounds
with a sufficient amount of the desired base, either neat or in a suitable inert solvent.
Examples of pharmaceutically acceptable base addition salts include sodium,
20 potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt.
When compounds of the present invention contain relatively basic functionalities,
acid addition salts can be obtained by contacting the neutral form of such compounds
with a sufficient amount of the desired acid, either neat or in a suitable inert solvent.
Examples of pharmaceutically acceptable acid addition salts include those derived
25 from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic,
monohydrogencarbonic, phosphoric, monohydrogenphosphoric,
dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous
acids and the like, as well as the salts derived from relatively nontoxic organic acids
like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric,
30 lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric,
methanesulfonic, and the like. Also included are salts of amino acids such as arginate
and the like, and salts of organic acids like glucuronic or galactunoric acids and the
like (see, for example, Berge *et al.*, "Pharmaceutical Salts", *Journal of*

Pharmaceutical Science, 66:1-19 (1977)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

5 [0050] The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

10 [0051] In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch
15 reservoir with a suitable enzyme or chemical reagent.

[0052] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple
20 crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0053] Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric
25 isomers and individual isomers are encompassed within the scope of the present invention.

[0054] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive
30 isotopes, such as for example tritium (^3H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

[0055] "Pharmaceutically acceptable acid addition salt" as used herein refers to salts retaining the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0056] "Pharmaceutically acceptable base addition salts" as used herein refers to those salts derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from pharmaceutically acceptable organic nontoxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethylamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like.

[0057] The term "biological property" as used herein means an *in vivo* activity that is directly or indirectly performed by a compound or pharmaceutical composition of the invention that is often shown by *in vitro* assays. In the present invention, the biological property is neuroprotection, including the prophylaxis, treatment and/or cure of disorders of the nervous system.

[0058] The term "isomer" as used herein refers to a compound having the same number and kind of atoms and hence the same molecular weight as another compound, but differing in respect to the arrangement or configuration of the atoms of the compound (*e.g.* *cis* and *trans* isomers). The term "isomer" also includes stereoisomers, diastereoisomers, enantiomers or mixtures thereof. The D-isomer is preferred.

[0059] The term "substructure" as used herein refers to a portion of a chemical compound. For example, a single aromatic ring of a naphthalene structure is herein referred to as a substructure of the entire naphthalene molecule.

5 [0060] The term "hydrate" as used herein refers to the product of water with a compound of the invention such that the H-OH bond is not split. A compound of the invention may form more than one hydrate. However, the amount of water in a hydrate of the invention is such that the compound remains stable. Preferably, a hydrate of a compound of the invention contains about 0.1-10% water.

10 [0061] The term "prodrug" as used herein refers to a pharmacologically inactive derivative or precursor of a compound of the invention which upon biotransformation, either spontaneous or enzymatic, within an organism releases a compound of the invention as a pharmaceutically active drug. A prodrug derivative of a compound of the invention contain groups cleavable under metabolic conditions such as, for example, solvolysis under physiological conditions or enzymatic degradation.

15 According to the invention, a compound of the invention resulting from the biotransformation of its prodrug derivative are pharmaceutically active in vivo. Prodrug derivatives of a compound of the invention may be designated as single, double, triple, etc., corresponding to the number of biotransformation steps required to release the pharmaceutically active compound of the invention within the organism

20 and/or indicating the number of functionalities present in the prodrug derivative. Prodrugs often offer advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (see, Bundgard, Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam (1985) and Silverman, The Organic Chemistry of Drug Design and Drug Action, pp. 352-401, Academic Press, San Diego, CA, (1992)).

25 [0062] As used herein, the term "saccharide" may be used interchangeably with the term "carbohydrate" and refers to single simple sugar moieties or monosaccharides as well as combinations of two or more single sugar moieties or monosaccharides covalently linked to form disaccharides, oligosaccharides, and polysaccharides. The term "saccharide" also includes N-acetylated and N-deacetylated

30 derivatives of such monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Saccharides for use in the invention may be linear or branched. Examples of suitable monosaccharides include, but are not limited to, known aldoses

and ketoses (*i.e.* aldehyde and ketone derivatives of straight-chain polyhydroxy alcohols containing at least three carbon atoms) including, for example, glyceraldehyde, erythrose, threose, ribose (Rib), arabinose (Ara), xylose (Xyl), lyxose (Lyx), allose, altrose, glucose (Glc), mannose (Man), gulose, idose, galactose (Gal),
5 talose, dihydroxyacetone, erythrulose, ribulose, xylulose, psicose, fructose (Frc), sorbose, and tagatose. Other examples of suitable monosaccharides include, but are not limited to, fucose (Fuc), N-acetylneuraminic acid (also called sialic acid, NANA, or NAN (Sia)), N-acetylglucosamine (GlcNAc), and N-acetylgalactosamine (GalNAc). The cyclic hemiacetal and hemiketal forms of the monosaccharides are
10 contemplated within the defined term. Other examples of suitable saccharides include, but are not limited to, those illustrated in Figure 1.

[0063] As used herein, the term "disaccharide" refers to a saccharide composed of two monosaccharides linked together by a glycosidic bond. Examples of disaccharides include, but are not limited to, lactose (Lac) (glycosidic bond between
15 Gal and Glc), sucrose (Suc) (glycosidic bond between Frc and Glc), and maltose (Mal), isomaltose and cellobiose (glycosidic bond between Glc and Glc).

[0064] The term "oligosaccharide" includes an oligosaccharide that has a reducing end and a non-reducing end, whether or not the saccharide at the reducing end is in fact a reducing sugar. In accordance with accepted nomenclature, an
20 oligosaccharide is depicted herein with the non-reducing end on the left and the reducing end on the right. An oligosaccharide described herein may be described with the name or abbreviation for the non-reducing saccharide (*e.g.*, Gal), followed by the configuration of the glycosidic bond (α or β), the ring bond, the ring position of the reducing saccharide involved in the bond, and then the name or abbreviation of
25 the reducing saccharide (*e.g.*, GlcNAc). The linkage between two sugars may be expressed, for example, as 2,3,2-->3, 2-3, or (2,3).

[0065] The term "sphingoid," as used herein, includes sphingosines, phytosphingosines, sphinganines, ceramides, and the like. Both naturally occurring and synthetically produced compounds are included.

30 [0066] The term "glycosphingolipid" is a carbohydrate-containing derivative of a sphingoid or ceramide. The carbohydrate residue is attached by a glycosidic linkage to O-1 of the sphingoid.

[0067] The term "sialic acid" (abbreviated "Sia") refers to any member of a family of nine-carbon carboxylated sugars. The most common member of the sialic acid family is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-1-onic acid (often abbreviated as Neu5Ac, NeuAc, 5 or NANA). A second member of the family is N-glycolyl-neuraminic acid (Neu5Gc or NeuGc), in which the N-acetyl group of NeuAc is hydroxylated. A third sialic acid family member is 2-keto-3-deoxy-nonulosonic acid (KDN) (Nadano et al. (1986) J. Biol. Chem. 261: 11550-11557; Kanamori et al. (1990) J. Biol. Chem. 265: 21811-21819. Also included are 9-substituted sialic acids such as a 9-O-C₁-C₆ 10 acyl-Neu5Ac like 9-O-lactyl-Neu5Ac or 9-O-acetylNeu5Ac, 9-deoxy-9-fluoro-Neu5Ac and 9-azido-9-deoxy-Neu5Ac. For review of the sialic acid family, see, e.g., Varki (1992) Glycobiology 2:25-40; Sialic Acids: Chemistry, Metabolism and Function, R. Schauer, Ed. (Springer-Verlag, New York (1992). The synthesis and use of sialic acid compounds in a sialylation procedure is described in, 15 for example, international application WO 92/16640, published October 1, 1992.

[0068] As used herein, the term "linker" refers to any element, atom, molecule, that serves to join one portion of a molecule to another. Linkers are well known to those skilled in the art. Linkers can be mono- or multifunctional.

[0069] As used herein, the term "donor" refers to any molecule that serves to 20 donate or provide a monosaccharide for addition to a growing saccharide chain or acceptor molecule. Thus the sugar moiety serves as one part of a donor molecule. Generally, the monosaccharide moiety is transferred from the donor to an "acceptor," as defined herein, by means of an enzymatic reaction. Donor molecules include those known to those of skill in the art and will vary depending upon the desired 25 monosaccharide to be transferred.

[0070] As used herein, the term "contacting" or "contact" in relation to an enzyme and "donor" and an "acceptor" to form a growing saccharide chain means bringing the enzyme and donor into association with the "acceptor" or growing saccharide chain to affect the addition of a new monosaccharide unit to the acceptor or growing 30 saccharide chain.

[0071] As used herein, the term "acceptor" refers to a molecule capable of receiving a monosaccharide moiety from a donor, each as defined herein. An

“acceptor” may accept more than one monosaccharide such that a linear or branched “saccharide,” as defined above, can be formed. Thus, the term “acceptor” includes a molecule containing a growing saccharide chain.

5 [0072] As used herein, the term “non-immobilized” in reference to an “acceptor”, as defined herein, means that the acceptor is not affixed or bound to a substrate. For example, an acceptor that is in solution would be a “non-immobilized” acceptor.

[0073] The term “glycosyltransferase” as used herein refers to enzymes that catalyze the transfer of sugar moieties from activated donor molecules to specific acceptor molecules, each as defined herein, forming glycosidic bonds. Examples of
10 glycosyltransferases include, but are not limited to, galactosyltransferase, glucosyltransferase, fucosyltransferase, and GalNAc transferase. Further, glycosyltransferases may be classified according to the stereochemistries of the reaction substrates and products as either retaining, *i.e.*, leading to retention of the anomeric configuration (for instance UDP-glucose \rightarrow α -glucoside), or inverting, *i.e.*,
15 leading to inversion of the anomeric configuration (for instance UDP-glucose \rightarrow β -glucoside) (Sinnott, M.L. *Chem. Rev.*, **90**:1171-1202 (1990)). The classification groupings of families of glycosyltransferases is explained by Coutinho, P.M. & Henrissat, B. (1999) Carbohydrate-Active Enzymes server, which can be found on the Internet at <<afmb.cnrs-mrs.fr/~pedro/CAZY/db.html>>.

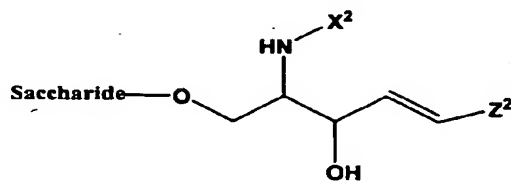
20 [0074] As used herein, the term “trans-sialidase” refers to an enzyme that catalyzes the addition of a sialic acid to galactose by means of an α -2,3 glycosidic linkage. Trans-sialidases may be found in many *Trypanosomyces* species and some other parasites. Trans-sialidases of these parasite organisms retain the hydrolytic activity of usual sialidase, but with much less efficiency, and catalyze a reversible
25 transfer of terminal sialic acids from host sialoglycoconjugates to parasite surface glycoproteins in the absence of CMP-sialic acid. *Trypanosome cruzi*, which causes Chagas disease, has a surface trans-sialidase the catalyzes preferentially the transference of α -2,3-linked sialic acid to acceptors containing terminal β -galactosyl residues, instead of the typical hydrolysis reaction of most sialidases (Ribeirão *et al.*,
30 *Glycobiol.*, **7**:1237-1246 (1997); Takahashi *et al.*, *Anal. Biochem.*, **230**:333-342 (1995); Scudder *et al.*, *J. Biol. Chem.*, **268**:9886-9891 (1993); Vandekerckhove *et al.*, *Glycobiol.*, **2**:541-548 (1992)). *T. cruzi* trans-sialidase (TcTs) has activity towards a

wide range of saccharide, glycolipid, and glycoprotein acceptors which terminate with a β -linked galactose residue, and synthesizes exclusively an α 2-3 sialosidic linkage (Scudder et al., supra). At a low rate, it also transfers sialic acid from synthetic α -sialosides, such as p-nitrophenyl- α -N-acetylneuraminic acid, but NeuAc2-3Gal β 1-4(Fuc α 1-3)Glc is not a donor-substrate. Modified 2-[4-methylumbelliferone]- α -ketoside of N-acetyl-D-neuraminic acid (4MU-NANA) and several derivatives thereof can also serve as donors for TcTs (Lee & Lee, *Anal. Biochem.*, **216**:358-364 (1994)). Enzymatic synthesis of 3'-sialyl-lacto-N-biose I has been catalyzed by TcTs from lacto-N-biose I as acceptor and 2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic as donor of the N-acetylneuraminil moiety (Vetere et al., *Eur. J. Biochem.*, **267**:942-949 (2000)). Further information regarding the use of trans-sialidase to synthesize α 2,3-sialylated conjugates can be found in European Patent Application No. 0 557 580 A2 and U.S. Patent No. 5,409,817. The intramolecular trans-sialidase from the leech *Macrobdella decora* exhibits strict specificity toward the cleavage of terminal Neu5Ac (N-acetylneuraminic acid) α 2 \rightarrow 3Gal linkage in sialoglycoconjugates and catalyzes an intramolecular trans-sialosyl reaction (Luo et al., *J. Mol. Biol.*, **285**:323-332 (1999)). Trans-sialidases primarily add sialic acid onto galactose acceptors, but will transfer sialic acid onto some other sugars. Transfer of sialic acid onto GalNAc, however, requires a sialyltransferase. Further information on the use of trans-sialidases can be found in PCT Application No. WO 93/18787; Vetere et al., *Eur. J. Biochem.*, **247**:1083-1090 (1997).

[0075] As used herein, the term "sialyltransferase" refers to enzymes that catalyze glycoside synthesis by inversion of the configuration of the added sugar and which require sugar nucleotides as the monosaccharide donor. An example of a sialyltransferase is the enzyme from the trypanosome *Trypanosoma rangeli* called TrSA (Buschiazzi et al., *EMBO J.*, **19**:16-24 (2000)).

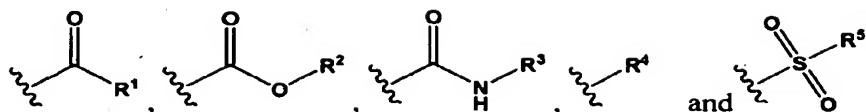
C. The Compositions

[0076] The invention provides a novel synthetic ganglioside according to formula (I):



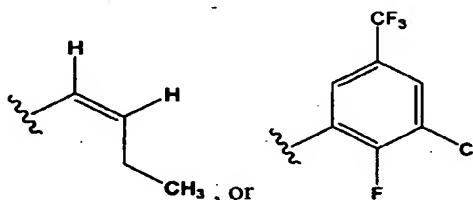
(I)

in which X^2 can be

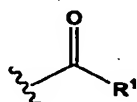


In the formulae above, R^1 , R^2 , R^3 , R^4 , and R^5 can be independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl. Z^2 can be substituted or unsubstituted alkenyl, substituted or unsubstituted pyridinyl, and substituted or unsubstituted phenyl.

[0077] The present invention also provides a novel ganglioside as described above, with the proviso that when R^1 is $-(\text{CH}_2)_{16}-\text{CH}_3$, then Z^2 is not unsubstituted pyridinyl,

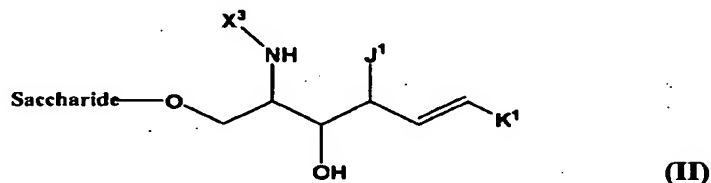


[0078] In another embodiment, X^2 can be

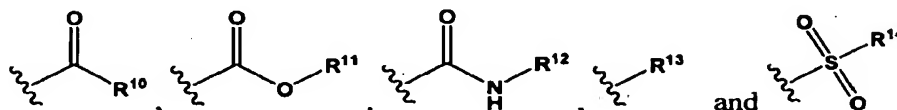


In the formula above, R^1 is substituted or unsubstituted alkyl. In yet another embodiment, R^1 can be a substituted or unsubstituted $\text{C}_8\text{--C}_{20}$ alkyl moiety. In still another embodiment, wherein R^1 can be haloalkyl. In another embodiment, R^1 can be a member selected from chloromethyl and dichloromethyl.

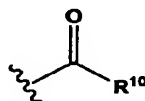
[0079] The invention further provides a novel synthetic ganglioside according to Formula (II):



in which X^3 can be

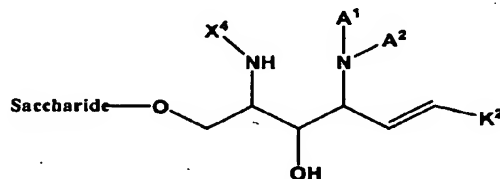


In the formulae above, R^{10} , R^{11} , R^{12} , R^{13} , and R^{14} can be members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl. J^1 can be $\text{---NR}^{15}\text{R}^{16}$, in which R^{15} and R^{16} can be members independently selected from substituted or unsubstituted alkyl. K^1 can be a member selected from substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. In another embodiment, at least one member selected from R^{15} and R^{16} can be $\text{---(CH}_2)_7\text{---CH}_3$. In a further embodiment, K^1 can be substituted or unsubstituted aryl. In yet another embodiment, K^1 can be substituted or unsubstituted phenyl. In still another embodiment, X^3 can be



In the formula above, R^{10} can be substituted or unsubstituted alkyl. In yet another embodiment, R^{10} can be a substituted or unsubstituted alkyl moiety. In another embodiment, R^{10} can be haloalkyl. In still another embodiment, R^{10} can be a member selected from chloromethyl and dichloromethyl.

[0080] The invention further provides a novel synthetic ganglioside according to Formula (III):

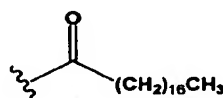


in which X^4 can be substituted or unsubstituted alkyl, $\text{---C(=M}^2\text{)R}^{20}$, $\text{---C(=M}^2\text{)---Q}^2\text{---R}^{20}$, $\text{---SO}_2\text{R}^{20}$. R^{20} can be a member selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. M^2 and Q^2 can be independently selected from O, NR^{21} and S. R^{21} can be H, substituted or unsubstituted alkyl, substituted or

unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocycloalkyl. A^1 and A^2 can be independently selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl. K^2 can be substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl.

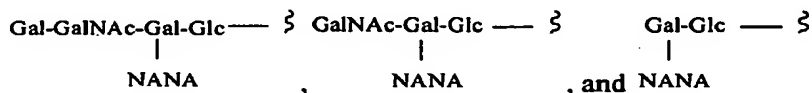
[0081] The present also provides a novel ganglioside compound as described above, with the proviso that if A^1 is $-(CH_2)_7-CH_3$, A^2 is $-(CH_2)_7-CH_3$, X^4 is $-C(=O)R^{20}$, and R^{20} is $-(CH_2)_{16}-CH_3$, then K^2 is not unsubstituted phenyl.

10 [0082] In another embodiment of the invention, X^4 can be $-C(=O)R^{20}$, and R^{20} can be substituted or unsubstituted alkyl. In yet another embodiment of the invention, X^4 can be

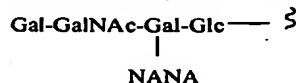


In yet another embodiment, A^1 and A^2 can be independently selected from substituted or unsubstituted alkyl. In still another embodiment, A^1 and A^2 can be $-(CH_2)_7-CH_3$. In another embodiment, K^2 can be substituted or unsubstituted aryl. In still another embodiment, K^2 can be phenyl.

[0083] In Formulae (I), (II), and (III), as set forth above, the saccharide can be a monosaccharide, a disaccharide, an oligosaccharide, a polysaccharide, an N-acetylated derivative thereof, and an N-deacetylated derivative thereof. In an exemplary embodiment, the saccharide is selected from the group consisting of:



[0084] In another exemplary embodiment, the saccharide is:



25 [0085] The invention also encompasses all pharmaceutically acceptable isomers, salts, hydrates, solvates, and prodrugs of each of the compounds described above. In addition, such compounds can exist in various isomeric and tautomeric forms, and all

such forms are meant to be included in the invention, along with pharmaceutically acceptable salts, hydrates, and solvates of such isomers and tautomers.

D. Methods of Making the Compositions

5 [0086] According to the invention, synthetic ganglioside compounds of formulae (I) (II) and (III) may be prepared using, unless otherwise indicated, conventional methods and protocols in chemistry and enzymology known in the art. For example, compounds of the invention may be prepared by synthetic and enzymatic processes as described in WO 03/017949 (published March 6, 2003) and WO 03/011879 (published February 13, 2003) which are herein incorporated by reference.

10 Compounds of the invention may be prepared by synthetic and enzymatic processes as outlined in Schemes 1-6 set forth below.

D1. Method of preparing the saccharide

[0087] The saccharide portion of the compounds of the invention may be prepared by any means known in the art including those methods described in U.S. Patents No. 5,922,577, 6,284,493 and 6,331,418, each of which is incorporated herein in its entirety by reference. Preferably, the saccharide portion of the compounds of the invention is prepared enzymatically whereby a specific enzyme may be used to affect transfer of a monosaccharide from a donor molecule to an acceptor molecule, each as defined herein.

20 [0088] More specifically, disaccharides, oligosaccharides and polysaccharides, as found in the synthetic ganglioside compounds of the invention, may be prepared biosynthetically by use of glycosyltransferases. Such glycosyltransferase reactions may be carried out in the presence of an organic solvent, such as, for example, methanol, ethanol, dimethylsulfoxide, isopropanol, tetrahydrofuran, chloroform, and the like, either singly or in combination. Alternatively, such glycosyltransferase reactions may be conducted in a biological medium *in vitro*, such as a biological buffer, a cell lysate, or on a chromatographic support, wherein the glycosyltransferase is immobilized on the chromatographic support and the other components of the reaction mixture are contacted with the glycosyltransferase by contacting the

25 components with the chromatographic support in an aqueous medium.

30

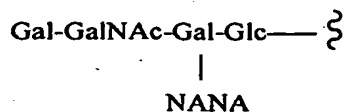
[0089] Glycosyltransferase-mediated synthesis of saccharides found in synthetic ganglioside compounds of the invention may also be conducted *in vivo*. For example,

whole-cell expression systems may be used for glycosyltransferase-mediated synthesis. Cell types that may be used for expression of glycosyltransferases and concomitant production of saccharide structures include bacterial cells, yeast cells, and insect cells, as would be understood by one of skill in the art. The desired
 5 saccharide product can be isolated from the cell in which it was synthesized by lysis of the cell, or by isolation of cell culture medium when using a cell that secretes the saccharide product into the culture medium. The saccharide product may then be purified by means described elsewhere herein, or it may be used without further purification in a lysate or cell culture medium.

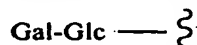
10 [0090] As would be understood by one of skill in the art, the enzyme used may vary depending upon the monosaccharide to be transferred. Examples of suitable enzymes include, but are not limited to, glycosyltransferases, trans-sialidases, and sialyltransferases. The choice of glycosyltransferase(s) used in a given synthesis method of the invention will depend upon the identity of the acceptor and donor
 15 molecules used as the starting material and the nature of the desired end product. A method of the invention can involve the use of more than one glycosyltransferase, where more than one saccharide is to be added. Multiple glycosyltransferase reactions can be carried out simultaneously, *i.e.*, in the same reaction mixture at the same time, or sequentially.

20 [0091] To obtain sufficient amounts of glycosyltransferase for large-scale *in vitro* reaction, a nucleic acid that encodes a glycosyltransferase can be cloned and expressed as a recombinant soluble enzyme by methods known to one of ordinary skill in the art. The expressed enzyme may then be purified by means known to one of ordinary skill in the art, or it may be used without further purification in a lysate or
 25 cell culture medium.

[0092] By way of example, the saccharide moiety:

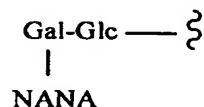


may be prepared by contacting an acceptor molecule containing a glucose (Glc) with a galactosyltransferase and a galactose (Gal) donor molecule to form:

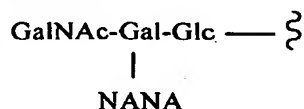


30

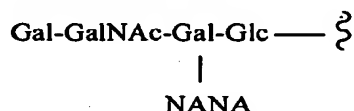
which in turn can be contacted with a trans-sialidase and a NANA donor molecule to form:



which in turn can be contacted with a N-acetylated galactose (GalNAc)-transferase and a GalNAc donor molecule to form:



which in turn can be contacted with a galactosyltransferase and a galactose (Gal) donor molecule to form the desired saccharide:



- 10 [0093] If the acceptor is a ceramide, the enzymatic step is typically preceded by hydrolysis of the fatty acid moiety from the ceramide; a fatty acid moiety can be reattached after completion of the glycosyltransferase reaction. The initial monosaccharide may be added, depending on the desired end product, either a ceramide glucosyltransferase (EC 2.4.1.80, for glucosylceramide) or a ceramide
- 15 galactosyltransferase (EC 2.4.1.45, for galactosylceramide). For review of glycosphingolipid biosynthesis, see, *e.g.*, Ichikawa and Hirabayashi, *Trends Cell Biol.*, 8:198-202 (1998). Ceramide glucosyltransferases are available from various sources. For example, the human nucleotide sequence is known (GenBank Accession No. D50840; Ichikawa *et al.*, *Proc. Nat'l. Acad. Sci. USA*, 93:4638-4643 (1996)), so
- 20 recombinant methods can be used to obtain the enzyme. The nucleotide sequence of the human ceramide galactosyltransferase also has been reported (GenBank Accession No. U62899; Kapitonov and Yu, *Biochem. Biophys. Res. Commun.*, 232: 449-453 (1997)), and thus the enzyme is easily obtainable. The acceptor used in these reactions can be any of N-acylsphingosine, sphingosine and dihydrosphingosine.
- 25 Suitable donor nucleotide sugars for the glycosyltransferase include UDP-Glc and CDP-Glc, while the galactosyltransferase typically uses UDP-Gal as a donor.

[0094] Methods of removing a fatty acid moiety from a glycosphingolipid are known to those of skill in the art. Standard carbohydrate and glycosphingolipid chemistry methodology can be employed, such as that described in, for example, Paulson *et al.*, *Carbohydrate Res.*, 137:39-62 (1985); Beith-Halahmi *et al.*, *Carbohydrate Res.*, 5: 25-30 (1967); Alais and Veyrieres, *Carbohydrate Res.*, 207:11-31 (1990); Grudler and Schmidt, *Carbohydrate Res.*, 135:203-218 (1985); Pongpipom *et al.*, *Tetrahedron Lett.*, 1717-1720 (1978); Murase *et al.*, *Carbohydrate Res.*, 188:71-80 (1989); Kameyama *et al.*, *Carbohydrate Res.*, 193:c1-c5 (1989); Hasegawa *et al.*, *J. Carbohydrate Chem.*, 10:439-459 (1991); Schwarzmunn and Sandhoff, *Meth. Enzymol.*, 138:319-341 (1987); Guadino and Paulson, *J. Am. Chem. Soc.*, 116: 1149-1150 (1994) (including supplemental material, which is also incorporated herein by reference). For example, hydrolysis of the fatty acid moiety can be effected by base hydrolysis. Once the glycosylation reactions are completed, the same or a different fatty acid can be attached to the product of the glycosylation reactions. Methods for coupling a fatty acid include those known in the art.

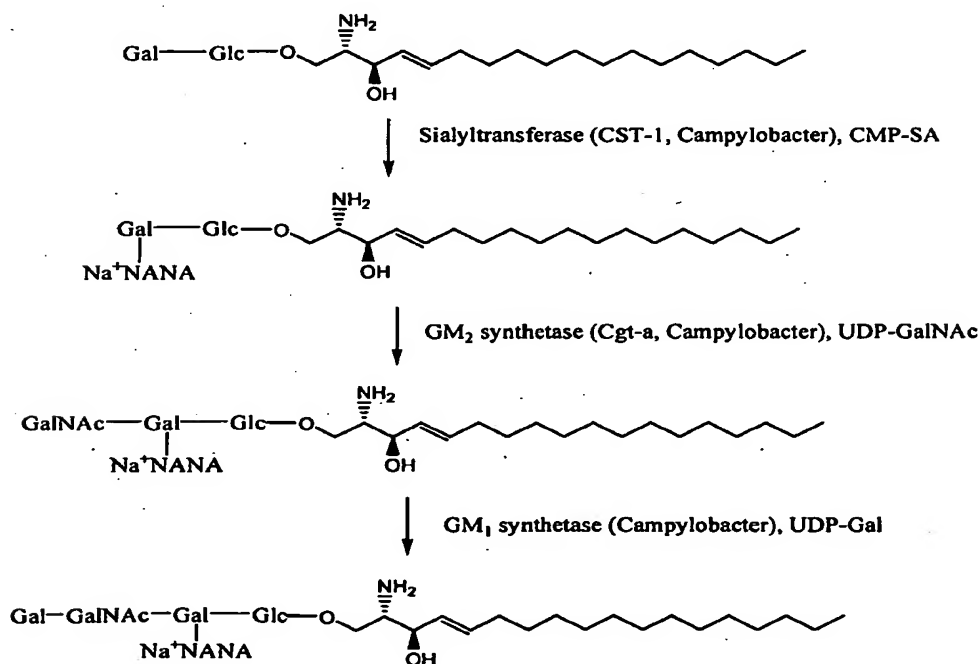
[0095] Another possible biosynthetic method for the synthesis of the saccharide portion of a compound of the invention is exemplified in Scheme 1 below. In a preferred embodiment, the acceptor molecule is non-immobilized. For example, the acceptor molecule may be free in solution or otherwise not associated with other acceptor molecules.

[0096] Additional saccharide residues may be added to a compound of the invention without prior modification of the glycosylation pattern of the glycosphingolipid starting material. Alternatively, the invention provides methods of altering the glycosylation pattern of a glycosphingolipid prior to adding the additional saccharide residues. If the starting glycosphingolipid does not provide a suitable acceptor for the glycosyltransferase which catalyzes a desired saccharide addition, one can modify the glycosphingolipid to include an acceptor by methods known to those of skill in the art. For example, to provide a suitable acceptor for a sialyltransferase, a suitable acceptor can be synthesized by using a galactosyltransferase to attach a galactose residue to, for example, a GalNAc or other appropriate saccharide moiety that is linked to the glycosphingoid. In other embodiments, glycosphingoid-linked oligosaccharides can be first "trimmed," either in whole or in part, to expose either an acceptor for the sialyltransferase or a moiety to

which one or more appropriate residues can be added to obtain a suitable acceptor. Enzymes such as glycosyltransferases and endoglycosidases are useful for the attaching and trimming reactions.

[0097] Sialyltransferases and other glycosyltransferases can be used either alone
 5 or in conjunction with additional enzymes. For example, Figure 2 shows a schematic diagram of two pathways for synthesis of the ganglioside GD₂ starting from lactosylceramide. Each pathway involves the use of two different sialyltransferases (an α 2,3ST and an α 2,8ST), as well as a GalNAc transferase. In the preferred pathway, the fatty acid is removed from the lactosylceramide by treatment with base (Step 1). Acetylation is then performed (Step 2), after which a sialic acid is attached to the galactose residue in an α 2,3 linkage by an α 2,3 sialyltransferase (Step 3). The sialylation steps are performed, preferably in the presence of an organic solvent as described herein, thereby driving the reaction nearly to completion. A GalNAc residue is then added to the galactose in a β 1,4 linkage using a GalNAc transferase
 10 (Step 5). Finally, a fatty acid is added, *e.g.*, by reaction with steroyl chloride, to complete the ganglioside (Step 6).

SCHEME 1

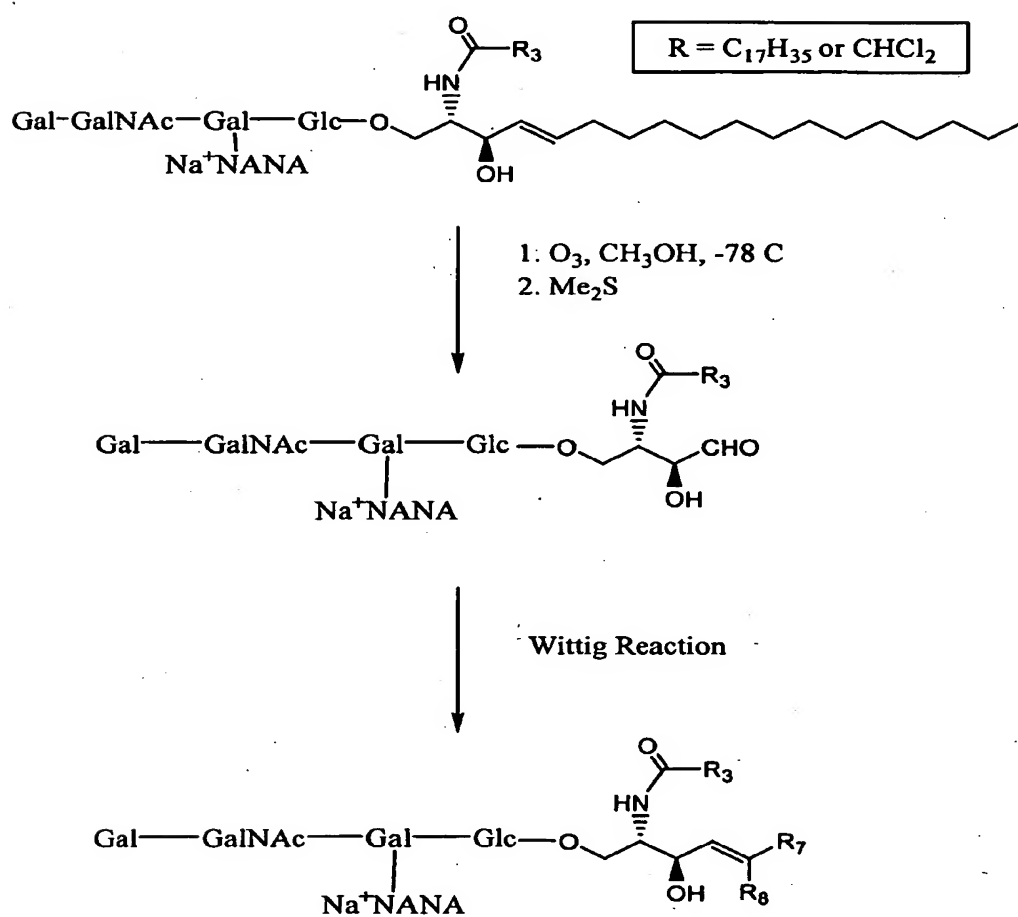


D2. Method of Preparing Compounds of Formulae (I) – (III)

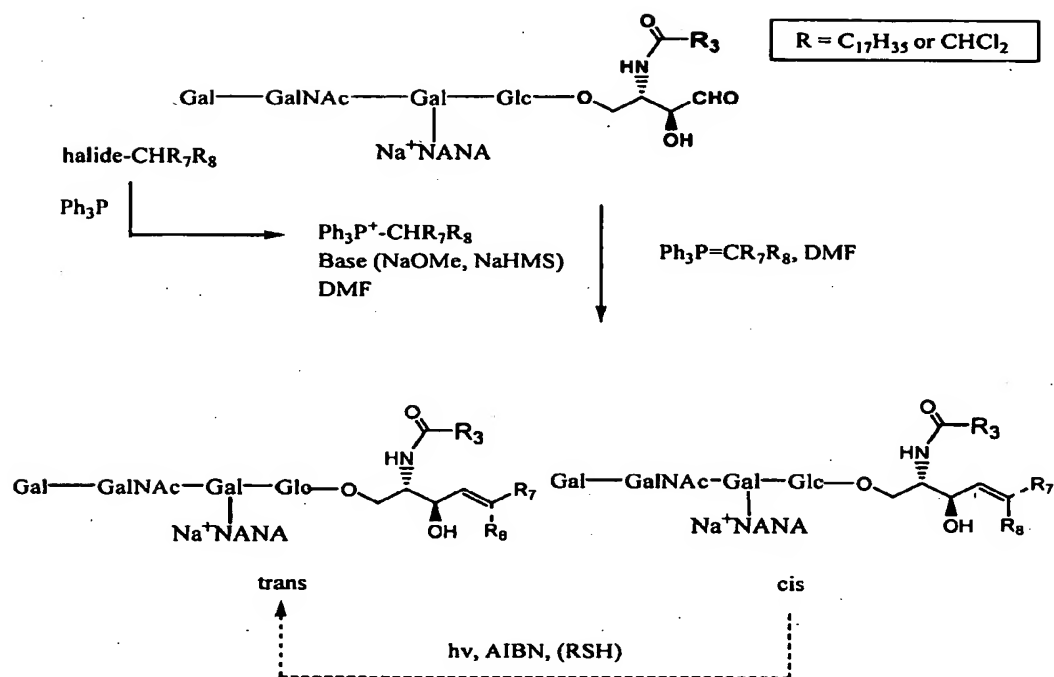
[0098] Compounds of the invention may be prepared by any means known in the art. Preferred synthetic pathways are illustrated in Schemes 2-5.

5

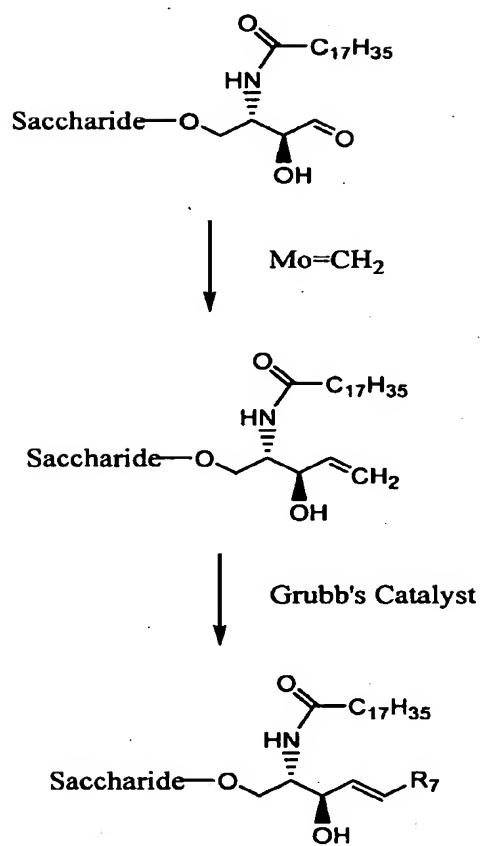
SCHEME 2



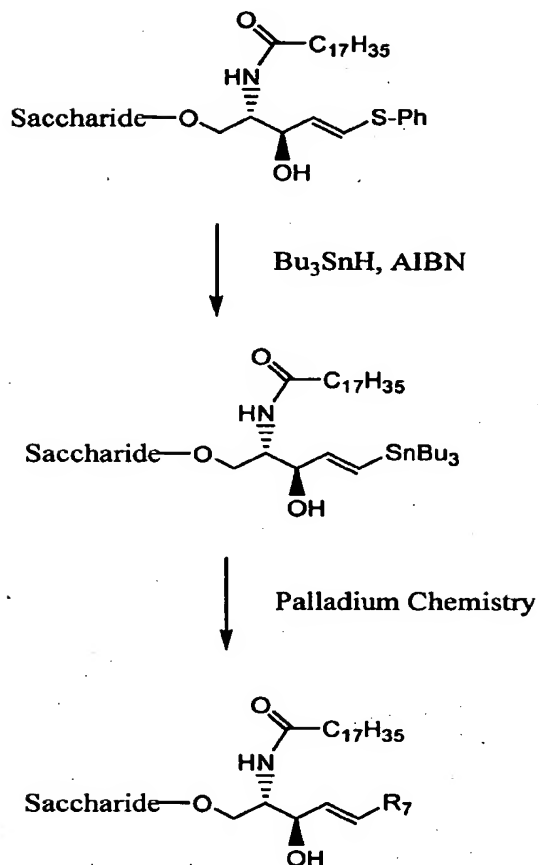
SCHEME 3



SCHEME 4



SCHEME 5



[0099] Once synthesized, the compounds of the invention may be isolated and purified by any means known in the art including, but not limited to, chromatography (e.g., thin, ion-exchange; column), filtration, membrane filtration (e.g., reverse osmotic membrane, nanfiltration), recrystallization, distillation, and the like.

[0100] A compound of the invention is useful in the field of neuroprotection. The term "neuroprotection" relates to any prophylaxis (pre-onset), treatment (on-set) and/or cure (post-onset) of indications resulting from the impairment or destruction of neuronal cells. Such indications include Parkinson's disease, ischemia, stroke, Alzheimer's, central nervous system disorders (e.g., spinal cord injury), multiple sclerosis, Huntington's disease, CABG, depression, anxiety, encephalitis, meningitis, amyotrophic lateral sclerosis, trauma, spinal cord injury, nerve injury, and nerve regeneration. A compound of the invention is also useful in the treatment of cancers.

in general, including liver, lung, colon, prostate, breast, pancreatic, and cancers of the brain, such as glioma and neuroblastoma. Further, a compound of the present invention is useful as an immunosuppressive and immunostimulatory agent, and has applications in organ transplantation, autoimmune disease, arthritis, Systemic Lupus
5 Erythematosus, irritable bowel disease, radiation toxicity and inflammation, psoriasis, dermatitis, multiple sclerosis, trauma and sepsis.

[0101] A compound of the invention can be used to stimulate or suppress T-cells and B-cells, and can be used for antibody suppression or stimulation. Methods of stimulating and suppressing T-cells and B-cells is well-known in the art. Further, a
10 compound of the invention may be used in a method to inhibit or activate membrane receptors, including G-protein coupled receptors, cell surface membrane receptor systems, and nuclear membrane receptors. A compound of the invention can further be used to treat type II diabetes and as an erythropoietin replacement.

[0102] A compound of the present invention is also useful as an inhibitor of
15 platelet aggregation. Further, a compound of the present invention is useful in AIDS treatment, by inhibiting viral adhesion through G-protein coupled receptors, including CCRC5 and CXCR4. A compound of the invention is also useful in the treatment of diseases such as Chagas disease, as well as diseases, disorders, and conditions described in U.S. Pat. Nos. 4,476,119, 4,593,091, 4,639,437, 4,707,469, 4,713,374,
20 4,716,223, 4,849,413, 4,940,694, 5,045,532, 5,135,921, 5,183,807, 5,190,925, 5,210,185, 5,218,094, 5,229,373, 5,260,464, 5,264,424, 5,350,841, 5,424,294, 5,484,775, 5,519,007, 5,521,164, 5,523,294, 5,677,285, 5,792,858, 5,795,869, and 5,849,717, each of which is incorporated by reference herein.

[0103] One possible mechanism of action of a compound of the invention is to
25 stimulate nerve growth factors. Another possible mechanism of action of a compound of the invention is to inhibit growth of cancer cells, and in particular, neuroblastoma cells. For example, it has been shown that administration of ganglioside GM3 to murine neuroblastoma cells can inhibit the growth of the neuroblastoma cells (Zhang *et al.*, *Anticancer Res.*, 15:661-666 (1995)). Ganglioside and ganglioside-like
30 compounds of the present invention can be used in a similar inhibitory capacity.

[0104] According to the invention, isolated and purified compounds of the invention for use in the field of neuroprotection or cancer treatment are of an

acceptable purity level. As would be understood by one of skill in the art, acceptable purity levels would depend upon the particular application. The compounds of the invention may be purified to levels ranging from about 80-100% pure, preferably, from about 90-100% pure, and more preferably about 95-100% pure.

5 **E. Pharmaceutical Compositions**

[0105] The invention further provides a pharmaceutical composition comprising at least one synthetic ganglioside compound of formulae (I), (II) and (III), each as set forth above, and a pharmaceutically acceptable carrier. Mixtures of synthetic gangliosides of the invention are also contemplated for use in pharmaceutical
10 compositions.

[0106] Pharmaceutical compositions of the invention may be prepared for storage or administration by any means known in the art. For example, a pharmaceutical composition of the invention may be prepared by mixing a compound of the invention, preferably having a desired degree of purity, with a pharmaceutically or
15 physiologically acceptable carriers or agent. The amount of active ingredient in these compositions is such that a suitable dosage in the range indicated is obtained.

[0107] A pharmaceutically acceptable carrier or agent may be any such carrier or agent known in the art. See, for example, in Gennaro *et al.*, Remington's Pharmaceutical Sciences, Mack Publishing Co., (1985). The pharmaceutical
20 composition of the invention may further include a binder (*e.g.*, acacia, corn starch or gelatin), an excipient (*e.g.*, microcrystalline cellulose), a disintegrating agent (*e.g.*, corn starch or alginic acid), a lubricant (*e.g.*, magnesium stearate), a sweetening agent (*e.g.*, sucrose or lactose), a buffer (*e.g.*, phosphate, citrate, acetate and other organic acid salts), an antioxidant (*e.g.*, ascorbic acid), a low molecular weight (less than
25 about ten residues) peptide (*e.g.* polyarginine), a protein (*e.g.*, serum albumin, gelatin, or immunoglobulins), a hydrophilic polymer (*e.g.*, polyvinylpyrrolidinone), an amino acid (*e.g.*, glycine, glutamic acid, aspartic acid, or arginine), a monosaccharide, a disaccharide, and other carbohydrates (*e.g.* cellulose or its derivatives, glucose, mannose or dextrans), a chelating agent (*e.g.*, EDTA), sugar alcohol (*e.g.*, mannitol or
30 sorbitol), a counterion (*e.g.*, sodium) and/or nonionic surfactants such as TWEEN, Plurionics or polyethyleneglycol. Additional acceptable adjuvants include those well

known in the pharmaceutical field, and as described, for example, in Gennaro *et al.*, Remington's Pharmaceutical Sciences, Mack Publishing Co., (1985).

[0108] A compound or a pharmaceutical composition of the invention may be administered in solid or liquid form depending upon the desired application. Thus, a
5 compound or pharmaceutical composition of the invention may be administered in solid form such as, for example, tablets, capsules, suppositories, in liquid form such as, for example, elixirs for oral administration, sterile solutions, sterile suspensions or injectable administration, and the like, or incorporated into shaped articles. A compound or a pharmaceutical composition of the invention may also be administered
10 as sustained release and timed release formulations. Other modes of administration of a compound or composition of the invention include, but not limited to, implantable medical devices (e.g., stents), inhalable formulations, sprays, transdermal, liposomes, gels, intracranial, and intrathecal.

[0109] A compound or pharmaceutical composition of the invention, especially
15 when administered in capsule form, may also contain a liquid carrier such as, for example, water, saline, or a fatty oil. Other materials of various types may be used as coatings or as modifiers of the physical form of the compound or pharmaceutical composition. For example, dissolution or suspension of the active compound of the invention in a vehicle such as an oil or a synthetic fatty vehicle like ethyl oleate, or
20 into a liposome may be desired.

[0110] According to the invention, such materials as well as compounds of the invention are nontoxic to the recipients at the dosages and concentrations employed, *i.e.* are pharmaceutically acceptable.

[0111] In general, a compound of the invention, alone or as part of a
25 pharmaceutical composition as described herein, may be used as a diagnostic or therapeutic agent for the prevention and/or treatment of disorders of the nervous system including neurological diseases such as, for example, Parkinson's disease, CABG, Alzheimer's Disease, and stroke. Further, a compound of the invention, alone or as part of a pharmaceutical composition as described herein, may be used as a
30 therapeutic agent for the treatment of certain types of cancer, including neuroblastoma.

[0112] Compounds and pharmaceutical compositions of the invention are suitable for use alone or as part of a multi-component treatment regimen in combination with other therapeutic or diagnostic agents such as, for example, other synthetic gangliosides of the invention, natural gangliosides, other synthetic gangliosides, anti-inflammatory compounds, analgesics, other neurotrophic factors (*e.g.*, growth factors). Coadministered compounds and agents may act in a synergistic fashion to enhance the neuroprotective activity of the compound of the invention.

[0113] The compounds and pharmaceutical compositions of the invention may be utilized *in vivo*, ordinarily in mammals such as primates, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*. The biological properties, as described above, of the compounds of the invention can be readily characterized by methods that are well known in the art including, for example, *in vitro* screening protocols and *in vivo* studies to evaluate the neuroprotective activity of the tested compound or pharmaceutical composition.

[0114] Subjects (animals or humans), preferably mammalian, in need of treatment may be administered a therapeutically effective amount, *i.e.*, a dosage that will provide optimal efficacy, of a compound of the invention, alone or as part of pharmaceutical composition. As would be recognized by those of skill in the art, a "therapeutically effective amount" and mode of administration will vary from subject to subject and thus will be determined on a case by case basis. Factors to be considered include, but are not limited to, the subject (*e.g.* mammal) being treated, its sex, weight, diet, concurrent medication, overall clinical condition, the particular compounds employed, and the specific use for which these compounds are employed. Therapeutically effective amounts or dosages may be determined by either *in vitro* or *in vivo* methods. In general, a "therapeutically effective amount" of a compound or composition is an amount that will result in the prophylaxis, treatment or cure of neuronal cell disorders. For example, a therapeutically effective amount of a compound or composition of the invention in the prophylaxis, treatment or cure of Parkinson's disease will be that amount that results in slower progression of the disease and/or development of motor skills. A therapeutically effective amount of a compound or composition of the invention in the prophylaxis, treatment or cure of Alzheimer's disease will be that amount that results in, for example, improvement of the subject's memory. A therapeutically effective amount of a compound or

composition of the invention in the prophylaxis, treatment or cure of the lasting effects of eschemia/stroke will be that amount that results in, for example, reduction of loss of neurological function (*e.g.*, speech, motor, etc.) and/or improvement of sympathetic or parasympathetic pathways.

- 5 **[0115]** Modes of administration include those known in the art including, but not limited to, oral, injection, intravenous (bolus and/or infusion), subcutaneous, intramuscular, colonic, rectal, nasal and intraperitoneal administration. Preferably, compounds of the invention, alone or as part of a pharmaceutical composition are taken orally.
- 10 **[0116]** For injection by hypodermic needle, it may be assumed the dosage is delivered into the body's fluids. For other routes of administration, the absorption efficiency may be individually determined for each compound of the invention by methods well known in pharmacology. Accordingly, as would be understood by one of skill in the art, it may be necessary for the therapist to titer the dosage and modify
- 15 the route of administration as required to obtain the optimal therapeutic effect. The determination of effective dosage levels, that is, the dosage levels necessary to achieve the desired result, will be within the ambit of one skilled in the art. Typically, a compound of the invention is administered at lower dosage levels, with dosage levels being increased until the desired effect is achieved.
- 20 **[0117]** A typical dosage might range from about 0.1 mg/kg to about 1000 mg/kg, preferably from 0.1 mg/kg to about 100 mg/kg, more preferably from about 0.1 mg/kg to about 30 mg/kg, more preferably from about 0.1 mg/kg to about 10 mg/kg, and more preferably 0.1 mg/kg to about 3 mg/kg. Advantageously, the compounds of the invention, alone or as part of a pharmaceutical composition, may be administered
- 25 several times daily, and other dosage regimens may also be useful. A compound of the invention may be administered on a regimen in a single or multidose (*e.g.* 2 to 4 divided daily doses) and/or continuous infusion.
- 30 **[0118]** A compound of the invention, alone or as part of a pharmaceutical composition, for administration may be sterilized prior to administration. Sterility may be readily accomplished by filtration through sterile membranes such as 0.2 micron membranes, or by other conventional methods. A compound of the invention, alone or as part of a pharmaceutical composition, typically may be stored in

lyophilized form or as an aqueous solution. pH may be a factor for certain modes of administration. In such instances, the pH typically will range between about 2-10, preferably, between about 5-8, more preferably 6.5-7.5, *i.e.*, physiological pH.

F. Methods of Using the Compositions in Screening Protocols

5 [0119] The compounds and pharmaceutical compositions of the invention may be utilized *in vivo*, ordinarily in mammals such as primates, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*. The effectiveness of the compounds of the invention as neuroprotective agents may be determined using screening protocols known in the art. For example, the biological properties, as
10 described above, of the compounds of the invention can be readily characterized by methods that are well known in the art including, for example, *in vitro* screening protocols (*e.g.* cell culture (MPTP (rat ventral mesophthalic cells), NMDA (mouse primary cortical neurons), ceramide (neuroblastoma-human)), CACO-2 (oral absorption), RBC lysis) and *in vivo* studies (*e.g.* mouse and primate MPTP toxicity
15 studies (IP, IV, and/or oral) for effectiveness in the treatment of Parkinson's, rat Stoke studies for effectiveness for treatment of neural damage due to stroke or CABG, and dog studies for treatment of CABG) to evaluate neuroprotective efficacy.

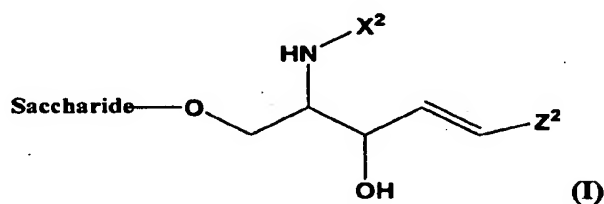
[0120] In the cell based assays, as described herein, the compounds of the invention exhibited 50-100% greater neuroprotective activity at lower concentrations
20 ranging between about 0.1 to about 1 μ M.

[0121] The invention is now described with reference to the following Example. This Example is provided for the purpose of illustration only and the invention should in no way be construed as being limited to this Example, but rather should be construed to encompass any and all variations which become evident as a result of the
25 teaching provided herein.

[0122] It should be understood that the foregoing discussion and examples merely present a detailed description of certain preferred embodiments. It will be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All the patents,
30 journal articles and other documents discussed or cited above are herein incorporated in their entirety by reference.

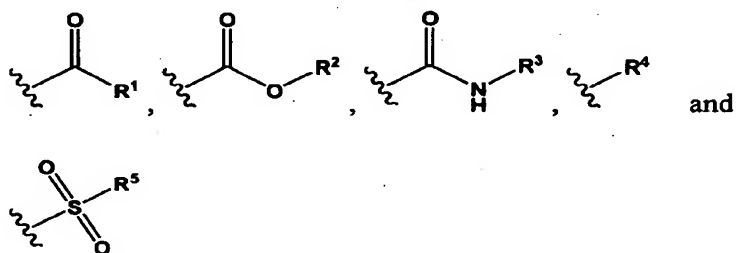
WHAT IS CLAIMED IS:

1. A compound having a structure according to Formula I:



wherein

X² is a member selected from

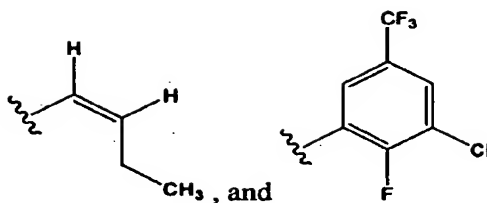


wherein

R¹, R², R³, R⁴, and R⁵ are members independently selected from substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted arylalkyl, and substituted or unsubstituted heteroarylalkyl;

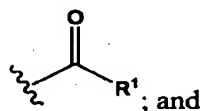
Z² is a member selected from substituted or unsubstituted alkenyl, substituted or unsubstituted pyridinyl, and substituted or unsubstituted phenyl;

with the proviso that when R¹ is $-(CH_2)_{16}-CH_3$, then Z² is not unsubstituted pyridinyl,



18

- 1 2. The compound according to claim 1, wherein X^2 is



2

- 3 R^1 is substituted or unsubstituted alkyl.

- 1 3. The compound according to claim 2, wherein R^1 is a
2 substituted or unsubstituted C_8 - C_{20} alkyl moiety.

- 1 4. The compound according to claim 2, wherein R^1 is haloalkyl.

- 1 5. The compound according to claim 4, wherein R^1 is a member
2 selected from chloromethyl and dichloromethyl.

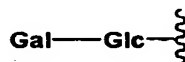
- 1 6. A pharmaceutical composition of claim 1 and a
2 pharmaceutically acceptable carrier.

- 1 7. A method for the prevention or treatment of a disorder of the
2 nervous system in an animal or human comprising the step of administering to an
3 animal or human in need thereof a therapeutically effective amount of the compound
4 of claim 1.

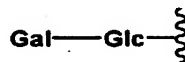
- 1 8. The method of claim 7, wherein said disorder of the nervous
2 system is selected from the group consisting of Parkinson's disease, ischemia, stroke,
3 Alzheimer's disease, depression, anxiety, encephalitis, meningitis, amyotrophic lateral
4 sclerosis, trauma, spinal cord injury, nerve injury, and nerve regeneration.

1 9. A method for the treatment of a glioma in a human comprising
2 the step of administering to the human in need thereof a therapeutically effective
3 amount of the compound of claim 1.

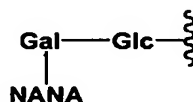
1 **10.** A method of synthesizing a synthetic ganglioside compound of
2 claim 1, wherein the steps of synthesis of the saccharide moiety comprise:
3 contacting an acceptor molecule comprising a sphingoid moiety and a
4 glucose (Glc) with a galactosyltransferase enzyme and a galactose (Gal) donor
5 molecule to form:



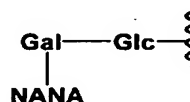
7 contacting the



9 with a transsialidase enzyme and a sialic acid (NANA) donor
10 molecule to form:



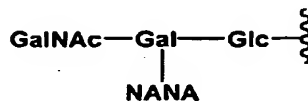
12 **contacting the**



with a N-acetyl galactose (GalNAc)-transferase enzyme and a
GalNAc donor molecule to form:



17 contacting the



19 with a galactosyltransferase enzyme and a galactose (Gal)
 20 donor molecule to form:

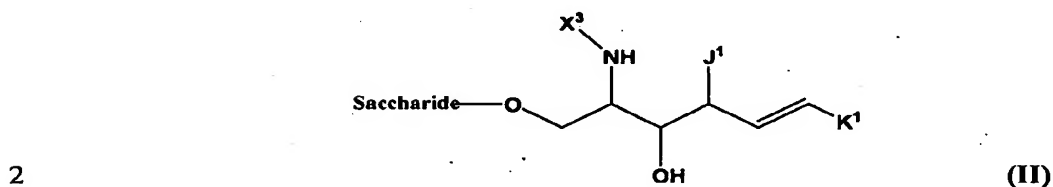


22 contacting the



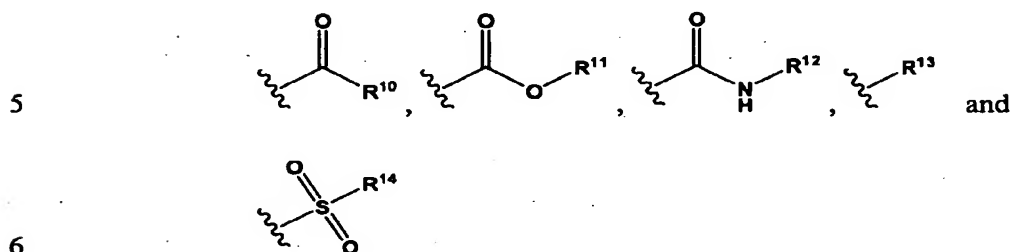
24 with a fatty acid moiety under conditions sufficient to form a
 25 ganglioside.

1 11. A compound having a structure according to Formula II:



3 wherein

4 X³ is a member selected from



7 wherein

8 R¹⁰, R¹¹, R¹², R¹³, and R¹⁴ are members independently selected from
 9 substituted or unsubstituted alkyl, substituted or unsubstituted
 10 aryl, substituted or unsubstituted heteroaryl, substituted or

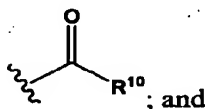
11 unsubstituted arylalkyl, and substituted or unsubstituted
12 heteroarylalkyl.
13 J^1 is $-NR^{15}R^{16}$
14 wherein
15 R^{15} and R^{16} are members independently selected from
16 substituted or unsubstituted alkyl; and
17 K^1 is a member selected from substituted or unsubstituted aryl and
18 substituted or unsubstituted heteroaryl.

1 12. The compound according to claim 11, wherein at least one
2 member selected from R^{15} and R^{16} is $-(CH_2)_7-CH_3$.

1 13. The compound according to claim 11, wherein K^1 is substituted
2 or unsubstituted aryl.

1 14. The compound according to claim 13, wherein K^1 is substituted
2 or unsubstituted phenyl.

1 15. The compound according to claim 11, wherein X^3 is



2
3 R^{10} is substituted or unsubstituted alkyl.

1 16. The compound according to claim 15, wherein R^{10} is a
2 substituted or unsubstituted alkyl moiety.

1 17. The compound according to claim 15, wherein R^{10} is haloalkyl.

1 18. The compound according to claim 17, wherein R^{10} is a member
2 selected from chloromethyl and dichloromethyl.

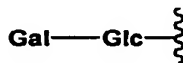
1 **19. A pharmaceutical composition of claim 11 and a**
2 **pharmaceutically acceptable carrier.**

1 **20.** A method for the prevention or treatment of a disorder of the
2 nervous system in an animal or human comprising the step of administering to an
3 animal or human in need thereof a therapeutically effective amount of the compound
4 of claim 11.

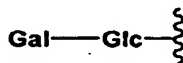
1 **21.** The method of claim 20, wherein said disorder of the nervous
2 system is selected from the group consisting of Parkinson's disease, ischemia, stroke,
3 Alzheimer's disease, depression, anxiety, encephalitis, meningitis, amyotrophic lateral
4 sclerosis, trauma, spinal cord injury, nerve injury, and nerve regeneration.

1 **22.** A method for the treatment of a glioma in a human comprising
2 the step of administering to the human in need thereof a therapeutically effective
3 amount of the compound of claim 11.

1 23. A method of synthesizing a synthetic ganglioside compound of
2 claim 11, wherein the steps of synthesis of the saccharide moiety comprise:
3 contacting an acceptor molecule comprising a sphingoid moiety and a
4 glucose (Glc) with a galactosyltransferase enzyme and a galactose (Gal) donor
5 molecule to form:



7 **contacting the**



9 with a transsialidase enzyme and a sialic acid (NANA) donor
10 molecule to form:



12



14



17



19



22



24

1

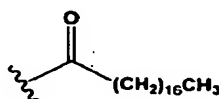


3

4 X^4 is substituted or unsubstituted alkyl, $-C(=M^2)R^{20}$, $-C(=M^2)-Q^2-R^{20}$,
 5 $-SO_2R^{20}$,
 6 wherein R^{20} is a member selected from substituted or
 7 unsubstituted alkyl and substituted or unsubstituted
 8 heteroalkyl;
 9 M^2 and Q^2 are members independently selected from O, NR^{21}
 10 and S;
 11 wherein
 12 R^{21} are members independently selected from H,
 13 substituted or unsubstituted alkyl, substituted or
 14 unsubstituted heteroalkyl, substituted or
 15 unsubstituted aryl, substituted or unsubstituted
 16 heteroaryl, substituted or unsubstituted
 17 heterocycloalkyl; and
 18 A^1 and A^2 are members independently selected from H, substituted or
 19 unsubstituted alkyl, and substituted or unsubstituted
 20 heteroalkyl;
 21 K^2 is a member selected from substituted or unsubstituted alkyl,
 22 substituted or unsubstituted aryl, substituted or unsubstituted
 23 heteroaryl, substituted or unsubstituted arylalkyl, and
 24 substituted or unsubstituted heteroarylalkyl;
 25 with the proviso that if A^1 is $-(CH_2)_7-CH_3$, A^2 is $-(CH_2)_7-CH_3$, X^4 is
 26 $-C(=O)R^{20}$, and R^{20} is $-(CH_2)_{16}-CH_3$, then K^2 is not
 27 unsubstituted phenyl.

1 25. The compound according to claim 24, wherein X^4 is
 2 $-C(=O)R^{20}$, and R^{20} is substituted or unsubstituted alkyl.

1 26. The compound according to claim 25, wherein X^4 is



2

- 1 27. The compound according to claim 24, wherein A¹ and A² are
2 independently selected from substituted or unsubstituted alkyl.
- 1 28. The compound according to claim 24, wherein A¹ and A² are
2 -(CH₂)₇-CH₃.
- 1 29. The compound according to claim 24, wherein K² is substituted
2 or unsubstituted aryl.
- 1 30. The compound according to claim 29, wherein K² is phenyl.
- 1 31. A pharmaceutical composition of claim 24 and a
2 pharmaceutically acceptable carrier.
- 1 32. A method for the prevention or treatment of a disorder of the
2 nervous system in an animal or human comprising the step of administering to an
3 animal or human in need thereof a therapeutically effective amount of the compound
4 of claim 24.
- 1 33. The method of claim 32, wherein said disorder of the nervous
2 system is selected from the group consisting of Parkinson's disease, ischemia, stroke,
3 Alzheimer's disease, depression, anxiety, encephalitis, meningitis, amyotrophic lateral
4 sclerosis, trauma, spinal cord injury, nerve injury, and nerve regeneration.
- 1 34. A method for the treatment of a glioma in a human comprising
2 the step of administering to the human in need thereof a therapeutically effective
3 amount of the compound of claim 24.
- 1 35. A method of synthesizing a synthetic ganglioside compound of
2 claim 24, wherein the steps of synthesis of the saccharide moiety comprise:
3 contacting an acceptor molecule comprising a sphingoid moiety and a
4 glucose (Glc) with a galactosyltransferase enzyme and a galactose (Gal) donor
5 molecule to form:


Gal—Glc— 

contacting the

Gal—Glc—

with a transsialidase enzyme and a sialic acid (NANA) donor molecule to form:

molecule to form:


Gal—Glc—
|
NANA

contacting the

Gal—Glc—

with a N-acetyl galactose (GalNAc)-transferase enzyme and a GalNAc donor molecule to form:

GalNAc donor molecule to form:

GalNAc—Gal—Glc—

contacting the

$$\text{GalNAc} - \underset{\substack{| \\ \text{NANA}}}{\text{Gal}} - \text{Glc} - \text{...}$$

with a galactosyltransferase enzyme and a galactose (Gal) donor molecule to form:

donor molecule to form:

Gal—GalNAc—Gal—Glc—

NANA

contacting the

$$\text{Gal}-\text{GalNAc}-\underset{\substack{| \\ \text{NANA}}}{\text{Gal}}-\text{Glc}-$$

with a fatty acid moiety under conditions sufficient to form a ganglioside.

ganglioside.

1

GLYCOLIPIDS

2

ABSTRACT OF THE DISCLOSURE

3 Novel synthetic gangliosides and pharmaceutical compositions containing such
4 synthetic gangliosides are described. Methods of making the novel synthetic
5 ganglioside compounds and compositions as well as their use in the field of
6 neuroprotection is also described.

I-SF/7167128.1

Application Data Sheet

Application Information

Application number::

Filing Date::

11/09/04

Application Type::

Provisional

Subject Matter::

Utility

Suggested classification::

Suggested Group Art Unit::

CD-ROM or CD-R??::

Number of CD disks::

Number of copies of CDs::

Sequence Submission::

Computer Readable Form (CRF)?::

Number of copies of CRF::

Title::

GLYCOLIPIDS

Attorney Docket Number::

40853-01-5157-PR

Request for Early Publication::

No

Request for Non-Publication::

No

Suggested Drawing Figure::

Total Drawing Sheets::

Small Entity?::

Yes

Latin name::

Variety denomination name::

Petition included?::

No

Petition Type::

Licensed US Govt. Agency::

Contract or Grant Numbers One::

Secrecy Order in Parent Appl.::

No

Applicant Information

Applicant Authority Type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Shawn
Middle Name::	
Family Name::	DeFrees
Name Suffix::	
City of Residence::	North Wales
State or Province of Residence::	PA
Country of Residence::	US
Street of Mailing Address::	126 Filly Drive
City of Mailing Address::	North Wales
State or Province of mailing address::	PA
Country of mailing address::	US
Postal or Zip Code of mailing address::	19454
Applicant Authority Type::	Inventor
Primary Citizenship Country::	US / Peoples Republic of China
Status::	Full Capacity
Given Name::	Zhi-Guang
Middle Name::	
Family Name::	Wang
Name Suffix::	
City of Residence::	Dresher
State or Province of Residence::	PA
Country of Residence::	US
Street of Mailing Address::	1473 Golden Drive
City of Mailing Address::	Dresher
State or Province of mailing address::	PA
Country of mailing address::	US

Postal or Zip Code of mailing address:: 19015

Correspondence Information

Correspondence Customer Number:: 43850

Representative Information

Representative Customer Number:: 43850

Domestic Priority Information

Application:: Continuity Type:: Parent Application:: Parent Filing Date::

Foreign Priority Information

Country:: Application number:: Filing Date::

Assignee Information

Assignee Name::

Street of mailing address::

City of mailing address::

State or Province of mailing address::

Country of mailing address::

Postal or Zip Code of mailing address::